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EFFECTS OF INUNDATION ON SIX VARIETIES OF TURFGRASS.(U)
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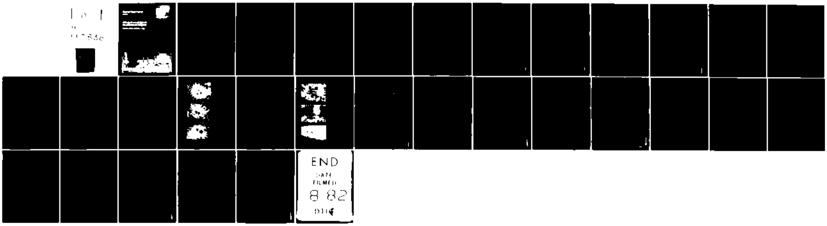

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Six cold-adapted grasses (manchar brome, boreal red fescue, durar fescue, nugget Kentucky bluegrass, meadow foxtail and sydsport bluegrass) were given ten-day dark and inundation stress treatments. Nugget Kentucky bluegrass grown in soil or gravel exhibited the best survival. Sydsport bluegrass did well in gravel. Meadow foxtail and manchar brome survived the treatments when grown in silt soil, but did not when grown on gravel soil. Rhizomes were regenerated by most of the grasses. Root transverse sections did not show any stress-related damage, but leaf sections did. The damage in the sections paralleled that

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observed macroscopically. Electrophoretic analysis for the peroxidase enzyme complex showed significant banding pattern differences before external damage was visible. This technique may prove to be a diagnostic tool for determining stress damage. Seedlings of all grasses except sydsport bluegrass survived a 15-day inundation.

PREFACE

This report was prepared by Frederic H. Erbisch, Professor of Botany, and Karen Stark, Botanical Research Associate, of the Keweenaw Research Center, Michigan Technological University, Houghton, Michigan.

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These conversion factors include all the significant digits given in the conversion tables in the ASTM <u>Metric Practice Guide</u> (E 380), which has been approved for use by the Department of Defense. Converted values should be rounded to have the same precision as the original (see E 380).

Multiply	By	To obtain
inch	25.4*	millimetre
pound (mass)	0.4535924	kilogram
acre	4,046873	metre^2

*Exact.

EFFECTS OF INUNDATION ON SIX VARIETIES OF TURFGRASS

Frederic H. Erbisch and Karen L. Stark

INTRODUCTION

The development of the Chena River Dam was necessary to prevent the type of flooding that occurred on 13 August 1967 in Fairbanks, Alaska. The dam has been built to temporarily retain Chena River flood waters and to slowly dissipate these waters over a ten-day period. The area immediately behind the dam has been cleared and graded to allow the trapped flood waters to flow properly. The cleared and graded flood plain is to be vegetated to prevent erosion and to maintain the proper grade. Woody vegetation, while able to stabilize the soil, would, because of its size, change the planned flow pattern of the flood waters. Therefore, the most suitable vegetation for this plain is a turfgrass, because its tight root structure prevents soil erosion and its herbaceous vegetative body does not impede the flow of water.

While various research groups have determined the suitability of various grasses in cold regions, none has presented data on survivability during and after inundation. Thus, the selection of a grass for flood plain planting is difficult. Many grasses could survive the harsh climate of the area but may not be able to survive a ten-day inundation period. The death of a grass would allow erosion to take place, which then could severely damage the flood plain.

Consequently, a laboratory study was initiated to determine the survivability of six cold-adapted grasses after a ten-day inundation period. In addition, a physiological analysis was done in an attempt to find an indicator test which could be used to determine the condition of a recently submerged grass.

Before the beginning of the study an extensive literature search was done to find other relevant studies (Appendix A). It was found that no in-depth physiological studies had been done with inundated grasses. Many of the papers simply described the survivability of grasses after various periods of inundation.

Next, turfgrass experts at several universities were contacted. They could not provide any information relating to physiological studies of inundated grasses.

Because of the lack of information on the physiological effects of inundation, the experiment was based on physiological systems that are affected by other types of stress, such as drought, mineral nutrient excess, and high temperatures. These include peroxidase, malic dehydrogenase, and acid and alkaline phosphatase enzyme systems.

MATERIALS AND METHODS

Grasses

Six grasses were used in the study: manchar brome, meadow foxtail, durar fescue, boreal red fescue, nugget Kentucky bluegrass and sydsport bluegrass. They were started in 4.5-inch plastic pots on 3 October 1978 at the Cold Regions Research and Engineering Laboratory in Hanover, New Hampshire. Sydsport bluegrass, boreal red fescue and durar fescue were reseeded during the week of 6 November 1978. Five of the grasses were planted on coarse gravel soil and topsoil; sydsport bluegrass was planted only on gravel soil (Table 1). The soils were from a mixture of samples taken near the flood control project in North Pole, Alaska (latitude 64°47'47", longitude 147°11'56"). A 1.2-cm layer of Ottawa sand was placed on the bottom of each pot, and the remainder of the pot was filled with either gravel or topsoil. Just before seeding, each pot was fertilized

Table 1. Planting regime for grasses used in the inundation study. Twelve pots of each soil type were planted for each type of grass.

Grass	Soil		Rate of seeding	
	Topsoil	Gravel	(lb/acre)	(g/pot)
manchar brome	X	X	100	0.10
meadow foxtail	X	X	120	0.11
durar fescue	X	X	160	0.15
boreal red fescue	X	X	160	0.15
nugget Kentucky bluegrass	X	X	120	0.11
sydsport bluegrass		X	120	0.11

with 15-15-15 fertilizer at the rate of 533 lb/acre or 0.43 g/pot.* The grasses were transported to Houghton, Michigan, on 11 December 1978 and placed in the greenhouse at Michigan Technological University. In addition to the grasses listed above, twelve pots of turfgrass (common Kentucky bluegrass and pennlawn red fescue) were sent to use in developing test procedures. This grass was taken from sod plots established at CRREL on 2 September 1977.

On 12 January and 15 June 1978 all pots were refertilized (15-15-15) at the rate of 0.43 g/pot. Because of bad weather, access to the greenhouse was blocked for several days, and many of the plants dried out. Several pots of meadow foxtail and manchar brome did not recover; these pots were reseeded on 13 January 1978.

Stress treatments and survivability analysis

Two 30-gal. garbage cans were used as dark chambers and two as inundation chambers. The inundation chambers were filled with tap water and allowed to stand for several days until the temperature stabilized. Then plants were placed in the water-filled chamber. The water level was approximately 60 cm above the top of the pots. During the ten-day inundation period each of the chambers was aerated to prevent anaerobic conditions from developing. The inundation chambers were illuminated for 18 hours per day with a bank of eight fluorescent GroLites. The illumination period was chosen as an average daylength during the time when flooding might occur.

The temperature of the water in these experiments was approximately 10°C, which is similar to that of a summer flood. The grasses, which were actively growing, also approximated summer conditions. Actively growing grasses covered with warm water are much more susceptible to inundation damage than are dormant grasses covered by cold (1-5°C) water (Bolton and McKenzie 1946, Kramer 1951, Beard 1973).

Three pots of each grass on each soil type were placed in the inundation chambers. Corresponding sets of plants were placed in the dark chambers to determine the stress effects of darkness. The control plants, which were placed around the outside of the inundation chambers, received 18 hours of artificial light per day. The remainder of the plants were

*Personal communication with S. Bigl of CRREL.

kept in the greenhouse. Water and air temperatures were taken at the beginning of the experiment. Thermocouples were placed on acrylic strips, so that temperature measurements could be made at the base of the pot and every 15 cm above the pots up to 60 cm.

After ten days the stressed plants were removed from the chambers, examined and returned to the greenhouse. These plants were observed over a ten-week recovery period. The control plants were used for comparison throughout the observation period.

The leaves of some of the stressed and control plants were trimmed to about 5-6 cm. Periodically, measurements of the leaves were made to determine the effect of the treatments on leaf growth.

The visual observations of the plants account only for the survival of the leaves. To determine if the rhizomes (the underground stems) survived, half of the plant and soil was removed from half of the pots. The holes were filled with commercial potting soil. If the rhizome had survived, new growth would eventually extend into the replacement soil and produce new leaves. These observations were made over a ten-week period.

Anatomical analysis

Root and leaf samples were taken from stressed and control plants and examined microscopically. Tissues were frozen and sectioned at 10 μm with a freezing microtome. Root and leaf sections were mounted in a drop of water, examined microscopically and photographed. Observations included general configuration, chloroplastid number and distribution, amount of melanin or brown pigment formations and cell disintegration.

Physiological analysis

It was decided to examine several enzyme systems to determine if any of these systems might provide an indication of inundation stress. The enzyme systems were picked because they had been shown to be affected by stress (peroxidase), are part of an essential metabolic pathway (malate dehydrogenase), or are important in maintaining certain balances within a cell or tissue (acid or alkaline phosphatase).

Because of the amount of time needed to do the analyses, fresh materials could not always be used. Therefore, root and leaf tissues were frozen in the appropriate buffer and stored at -20°C until needed. Approximately 0.5 g of tissue was placed in 1 mL of buffer. (Earlier in-house studies had shown that freezing had no detrimental effect on isoenzymes.)

The plant materials were prepared for analysis by thawing them and placing them in a 10-mL mortar with a pinch of Ottawa sand; the mixture was crushed and ground with a pestle. To prevent denaturation of the enzymes, the mortar and the enzyme solution were placed in an ice bath until needed. No phenol inhibitor was added to the enzyme solution.

The leaf and root extract samples were analyzed by starch gel electrophoresis. This method was chosen because of its sensitivity and its ability to separate different forms of an enzyme system. Although overall enzymatic activity may be the same after a stress, the composition of the system may be different.

In the electrophoresis technique an enzyme-containing solution is applied to one point on a starch gel. Then a low-amperage electric current is passed through the gel. The enzyme, which is a charged molecule, migrates along the electrical gradient toward either the cathode or the anode, depending on its charge. Due to differences in their charges, molecules migrate at different rates in the electrical field.

The gel is removed from the field and placed in a specific enzyme substrate solution. The enzyme in the gel acts on the substrate, which is combined with a dye, and a colored deposit is formed at the site of the enzyme. Then the gel is placed in a fixative solution, which deactivates the enzyme and preserves the gel. The number of colored bands on the gel indicates the different forms of the specific enzyme complex. Variations in band number can indicate either genetic differences or adjustment reactions to other factors. In this study, where the strains of plants were uniform, the differences would be due to non-genetic factors (i.e. water and dark stresses).

Electrophoretic separations were performed in a 12% starch gel with a tris-citric-acid buffer system at pH 7. The gel was prepared by adding 200 mL of boiling gel buffer to 100 mL of cold gel buffer with 36 g of starch (Scandalios 1974, Strieleman 1979). The mixture was shaken vigorously and aspirated for approximately 15 seconds to remove trapped air. Then the gel was quickly poured into a shallow electrophoretic mold and allowed to set.

After the gel had solidified, it was cut so that the enzyme solution could be added. Filter paper wicks, which were approximately 3 x 10 mm pieces of #3 Whatman filter paper, were soaked in the prepared leaf or root solutions. The excess solution was removed by blotting them on a paper towel, and they were inserted in the cut on the gel (Fig. 1). The prepared

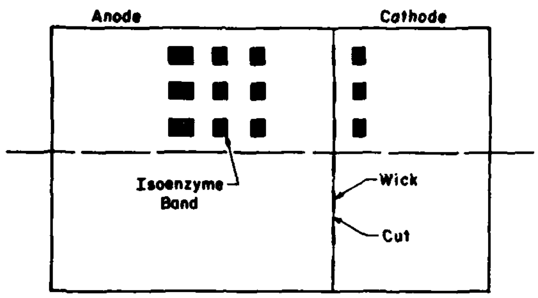


Figure 1. Diagram of an electrophoretic starch gel. The upper portion of the diagram represents a gel on which the various components of an enzyme complex have been separated and stained. The lower portion of the diagram represents a gel just prior to separation. The cut is the origin, where the enzyme-soaked wicks were placed.

gel was placed in the refrigerated electrophoresis chamber (4°C), and electrodes were attached. A 25-mA current was passed through the gel for three hours. Then the gel was cut into two or three thin slices, and each slice was placed in a staining solution. After 2-24 hours the gels were placed in a fixing solution, and the results were recorded. Each test was run a minimum of three times on different samples for each plant and stress treatment.

The recipes for the enzyme stains and buffers were developed by Shaw and Prasad (1970) and Selander et al. (1971). The recipe constituents and staining procedures are shown in Table 2.

Germination and seedling survival

In addition to the tests on mature plants a seedling survival test was done for each type of grass. First, a seed germination test was run, where 100 seeds were placed on filter paper wetted with distilled water. The plates were placed in a 25°C incubator and examined periodically for germination. This test was repeated a minimum of three times.

Then, seeds of each grass were placed on fine-grained Ottawa sand, 100 in each pot with six pots for each species. After the seedlings reached a length of 6-8 cm, half of the pots were placed in an inundation chamber for 15 days. The remainder of the plants served as controls. After inundations all of the seedlings were put into the greenhouse and observed.

Table 2. Recipes for starch gel electrophoresis enzyme stain and buffers (Shaw and Prasad 1970, Selander et al. 1971).

ACID PHOSPHATASE

Stain buffer

Na acetate$\cdot 3H_2O$	6.8 g
HCl (1N)	14.8 mL
H_2O to	1.0 L

Adjust pH with 0.1N HCl to 5.0

Stain

Na α-naphthyl acid phosphate	100 mg
Stain buffer	100 mL
Black K salt	100 mg

Incubate gel at 37°C until bands appear
Wash and fix
Gel slices should be soaked in the acid buffer before staining

PEROXIDASE

3-amino 9-ethyl carbazole	50 mg
Dimethyl formamide	5 mL
0.05 M Na acetate (pH 5.0)	92.5 mL
0.1 M $CaCl_2$	2 mL
3% H_2O_2	0.5 mL

Dissolve 3-amino 9-ethyl carbazole in dimethyl formamide before adding other ingredients
Add the 3% H_2O_2 just before staining.
Incubate gel in cold room until bands appear
Wash and fix

ELECTRODE BUFFER (pH 7.0)

Tris	16.35 g
Citric acid	9.04 g
H_2O	1 L

ALKALINE PHOSPHATASE

β-naphthyl Na phosphate	50 mg
Fast blue RR	50 mg
$MgSO_4 \cdot 7H_2O$	123 mg
H_2O	100 mL

Incubate at 37°C until blue bands appear
Wash and fix

MALATE DEHYDROGENASE

Stain

β-diphosphopyridine (NAD)	50 mg
Nitro blue tetrazolium	30 mg
Phenazine methosulfate	2 mg
1 M Na L-malate, pH 7.0 (substrate)	10 mL
0.5 M tris-HCl (pH 7.1)	15 mL
H_2O	
0.1M NaOH	70 mL
	5 mL

Substrate

L-malic acid	13.4 g
2 M $Na_2CO_3 \cdot H_2O$ (248 g/L)	49 mL
H_2O	1000 mL

Incubate at 37°C in the dark for 1 hour
Wash and fix

GEL BUFFER (pH 7.0)

Dilute 66.7 mL of electrode buffer to 1 L

FIXING SOLUTION

Acetic acid	10 mL
Methanol	50 mL
Water	50 mL

Table 3. Summary of survival, growth, rhizome regeneration and leaf transverse section data for six grasses given ten-day stress treatments. (Soil type: S = topsoil, G = gravel; Treatment: C = control, D = dark, I = inundation; Development: w.d. = well developed, p.d. = poorly . -veloped.)

Grass	Soil type	Treatment	Appearance of leaves after treatment: Immediately	Appearance of leaves after treatment: 10 weeks	Growth of leaves (cm)	Rhizome regeneration	Leaf transverse section data: Development	Leaf transverse section data: Section form	Leaf transverse section data: Chloroplastid color
Manchar brome	S	C	normal	normal	18	yes	w.d.	intact	green
	S	D	chlorotic	chlorotic, poor condition	18	no	w.d.	broken	light green
	S	I	normal	dead*	18†	yes	w.d.	intact	light green
	G	C	normal	normal	18	no	w.d.	intact	green
	G	D	chlorotic to black	dead*	18†	yes	p.d.	broken	brown
	G	I	chlorotic	all new growth, normal	18	no	p.d.	broken	light green
Boreal red fescue	S	C	normal	normal	20	yes	w.d.	intact	green
	S	D	chlorotic to brown	dead	0	no	p.d.	broken	light green
	S	I	normal	most normal	3	yes	w.d.	intact	green
	G	C	normal	normal	15	no	w.d.	intact	green
	G	D	chlorotic to brown	fair condition	15	yes	w.d.	intact	light green
	G	I	slight chlorosis	dead*	15†	no	w.d.	intact	light green
Durar fescue	S	C	normal	normal	11	yes	w.d.	intact	green
	S	D	chlorotic	chlorotic	11	no	w.d.	intact	light green
	S	I	normal	dead*	0	yes	w.d.	intact	green
	G	C	normal	normal	7	no	w.d.	intact	green
	G	D	brownish	dead	0	no	p.d.	intact	light green and brown
	G	I	chlorotic	dead	0	no	w.d.	intact	light green
Nugget Kentucky bluegrass	S	C	normal	normal	9	yes	w.d.	intact	green
	S	D	chlorotic	recovering, slightly chlorotic	9	yes	w.d.	intact	light green
	S	I	normal	normal	9	yes	w.d.	intact	green
	G	C	normal	normal	20	no	w.d.	intact	green
	G	D	chlorotic to brown	dead*	3-4	yes	p.d.	broken	light green to brown
	G	I	light green	dead*	20	yes	w.d.	broken	light green
Meadow foxtail	S	C	normal	normal	17	yes	w.d.	intact	green
	S	D	chlorotic to brown	recovering, green	17	no	—	—	—
	S	I	normal	normal	17	yes	w.d.	intact	green
	G	C	normal	normal	14	no	w.d.	intact	green
	G	D	brownish	most dead	14	no	p.d.	broken	brown
	G	I	normal	most dead	0	no	w.d.	intact	green
Sydsport bluegrass	G	C	normal	normal	8	yes	w.d.	intact	green
	G	D	chlorotic	normal	8	yes	w.d.	slightly broken	light green
	G	I	normal	normal	8	yes	w.d.	intact	green

* The leaves were dead at the end of the ten-week observation period, but new leaves were produced after this period.

† Most of the leaves were dead; the measurement reflects the growth of the few surviving leaves.

RESULTS AND DISCUSSION

Survival and growth

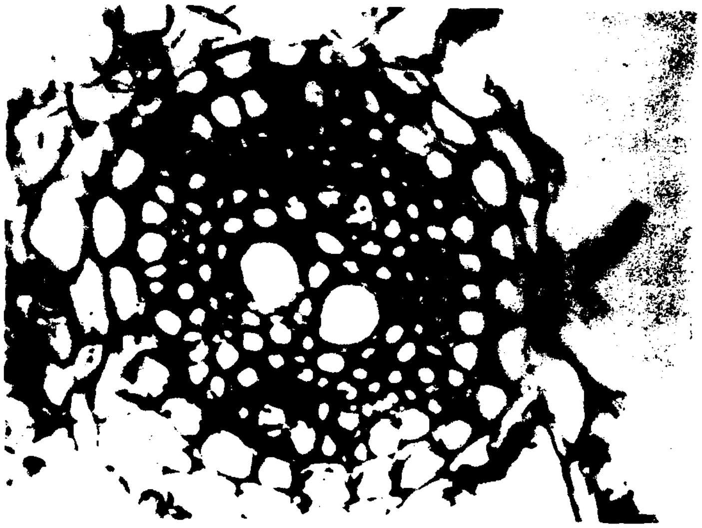
The results of the survival study are shown in Table 3. Nugget Kentucky bluegrass exhibited the best survival and growth of any of the topsoil-grown grasses; meadow foxtail was the second best and manchar brome the third. Sydsport bluegrass had the best survival and growth on gravel soil. Nugget Kentucky bluegrass was the only other grass to do well on gravel soil. Neither of the fescues did well on either of the soils.

Rhizomes, the underground stems of these grasses, grow through the soil. As they grow, roots and leaf buds are produced. Under normal conditions, the buds will produce the leaves, which grow through the soil and emerge into the atmosphere. If the rhizome is adversely affected by some agent such as inundation, bud and leaf production will also be adversely affected. The visible manifestation of this effect could be a decline of the mature leaves; leaves would become chlorotic and could die if the effect was severe enough. New leaf production would be limited or nonexistent. In this series of experiments the detrimental effects of the inundation were evidenced by the leaves; however, the effects on the rhizomes were not seen directly, but indirectly through the production or lack of production of new leaves (Table 3).

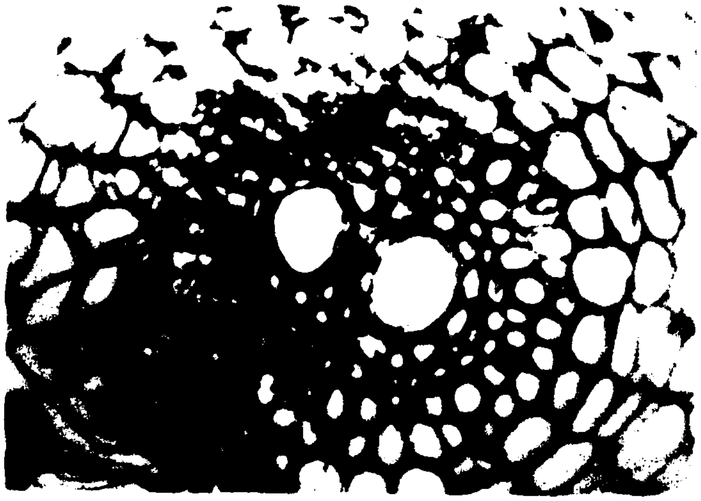
Manchar brome, nugget Kentucky bluegrass, and the fescues appeared to have initially survived the inundation treatment quite well, but within several weeks the leaves of these grasses had died. However, new leaves were produced by these plants, indicating that the roots and rhizomes had survived. If this had happended at the Chena Dam flood plain, these grasses would have prevented soil erosion, though the original leaves were dead.

Water-saturated soil has little oxygen in it, and this lack of oxygen adversely affects the roots (Childers and White 1942). This lack of soil oxygen may also affect the growth of the whole plant, as explained by Treshow (1970): "When the pore spaces of the soil are filled with water the plant root suffocates. The immediate effect of suffocation is suppression of growth. Plants may survive brief periods of oxygen deficiency where as little as 0.5% O_2 is available in the soil, but roots need 2-8% for optimum growth. Below this, leaves become chlorotic, growth ceases, no new roots develop, shoots die back, and death ensues. This decline and

a. Control root.



b. Dark-stressed root.



c. Inundation-stressed root.

Figure 2. Transverse sections of durar fescue roots.

death may take place in a few days in some plants, or months or even years in others."

Anatomical analysis

Several hundred transverse sections of roots and leaves were examined. No significant differences were seen in any of the roots (Fig. 2), even in those plants where the leaves had eventually died. Perhaps no differences were seen because the sections were made too soon after the stress treatment.

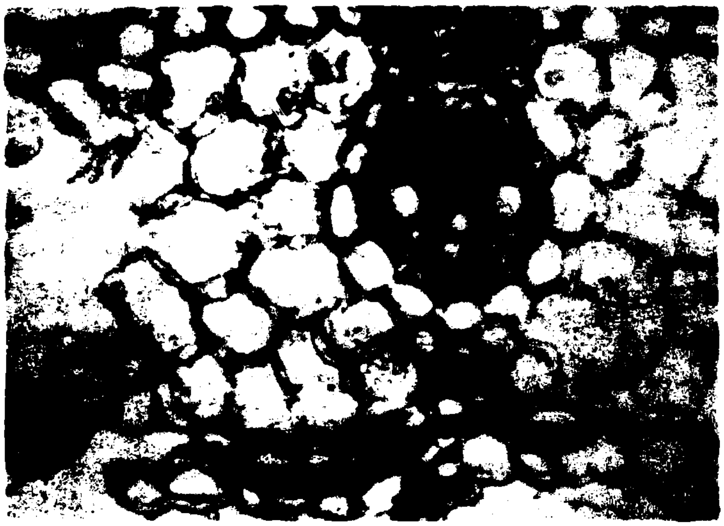
Differences were seen in leaf sections that were made immediately after the stress treatment. Those leaves that were chlorotic or brown had fewer chloroplastids in the mesophyll cells, and these chloroplastids were light green to brown (Table 3, Fig. 3). Although the leaf sections did show differences, this would not be a good diagnostic technique for assessing inundation damage, because the internal damage paralleled the external leaf appearance.

Physiological analysis

A shortage of plant material limited the number of tests and replicates that could be done. Because the acid and alkaline phosphatases did not show any significant differences, these tests were not run on all of the material or were not replicated. Differences in banding patterns were noted in the peroxidase and malic dehydrogenase tests. The results of these tests are shown in Figures 4 and 5. These results are the averages of the enzyme migrations for at least three replicate tests, with each test having three sets of enzyme runs.

Peroxidase catalyzes a reaction between hydrogen peroxide and many kinds of substrates. Under certain conditions peroxidase will react with compounds other than hydrogen peroxide, such as indoleacetic acid, phenylpyruvate, serine, alanine and tryptophane (Fric 1976). The physiological activity of peroxidase is not fully understood because of its wide range of activities. Also, the enzyme is found in many parts of the cell (nucleus, mitochondria, ribosomes and systems associated with cell wall formation), making it difficult to assign a specific role or activity for this enzyme (Fric 1976, Hahlbrock and Grisebach 1979). However, this enzyme system was felt to be important in this study because of its reactions to stress (Hare 1964, Gerloff et al. 1967, Ku et al. 1970, Krasnuk et al. 1975, Strieleman 1979). A reaction to stress is generally shown as either the addition or loss of a band or bands in the electrophoretic gel pattern.

a. Control leaf.



b. Dark-stressed leaf. The cells of this leaf had fewer chloroplastids than did the control leaves.

c. Inundation-stressed leaf. The cells of this leaf had no chloroplastids.

Figure 3. Transverse sections of grass leaves.

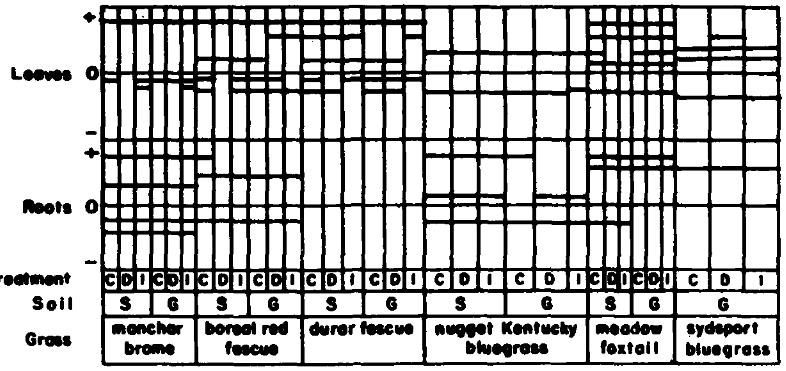


Figure 4. Leaf and root peroxidase isoenzyme banding patterns from control and stressed grasses. (+ = anode, o = origin, – = cathode, C = control, D = dark-stress treatment, I = inundation-stress treatment, S = topsoil, G = gravel.)

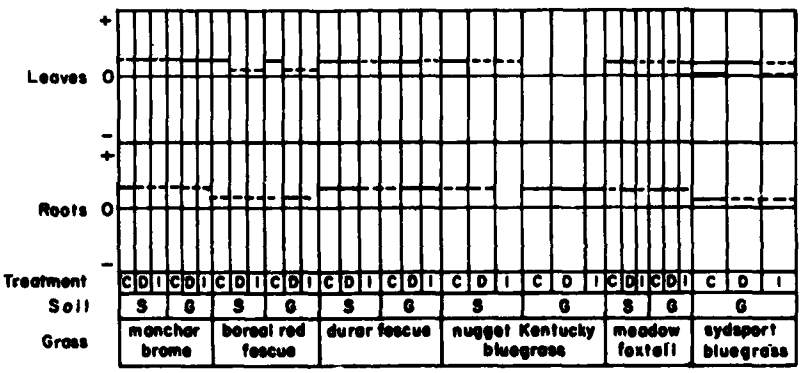


Figure 5. Leaf and root malic dehydrogenase isoenzyme banding patterns from control and stressed grasses. (+ = anode, o = origin, – = cathode, C = control, D = dark-stress treatment, I = inundation-stress treatment, S = topsoil, G = gravel soil, —— = darkly stained band, --- = lightly stained band.)

In the series of tests with the stressed grasses, peroxidase banding pattern differences were noted (Fig. 4). The inundation treatment affected the leaf peroxidase patterns of manchar brome on soil and gravel; boreal red fescue, durar fescue and nugget Kentucky bluegrass on gravel; and meadow foxtail on soil. Differences for roots were only shown by nugget Kentucky bluegrass grown on gravel. Dark stress treatments also affected the peroxidase banding, but not always in the same way as did the inundation stress (e.g. manchar brome on soil).

Malic dehydrogenase is the enzyme responsible for the conversion of malic acid to oxaloacetic acid and for reducing nicotinamide adenine dinu-

cleotide (Devlin 1975). This reaction is important in the production of biological energy. If this enzyme system is not functioning, the production of energy is severely limited; the energy reduction causes growth to stop and if severe enough, results in the death of the organism. Cytoplasmic malic dehydrogenase is also important in anabolic pathways such as gluconeogenesis.

The stressed grasses did exhibit differences in malic dehydrogenase patterns (Fig. 5), as well as differences in staining intensity. A lightly stained band indicates lower enzymatic activity than one that is darkly stained. All grasses except manchar brome exhibited some differences between control and stressed leaves, either in staining intensity or in the position of the bands. Some differences were seen in roots also, but only in staining intensity, not in band position. Differences in stain intensity were seen in the roots of manchar brome and durar fescue grown on soil and meadow foxtail grown on gravel.

Several correlations can be made between the enzymatic data and the survival data. First, the malic dehydrogenase complex is generally affected by the stress treatments given the grasses; however, there is no correlation between the survival of leaves and the enzyme variations. For example, similar isoenzyme differences were found in the leaves of meadow foxtail and durar fescue, but the leaves of durar fescue died while the leaves of meadow foxtail did not. Second, the effects of the stress treatments on root peroxidases appear to be insignificant with regard to the survival of the grasses. Third, peroxidase differences seen in banding patterns of leaves can be correlated with leaf survival in all cases except for meadow foxtail. Banding differences were noted in manchar brome, boreal red and durar fescues, and nugget Kentucky bluegrass, and the leaves of these plants died. When the leaves were analyzed for peroxidase activity, all were alive and at worst exhibited a slight chlorosis. Later, the leaves on some of these plants died. It is not known what relation exists between the change in banding pattern and the subsequent death of the leaf; further testing would be needed to ascertain this relation. It would appear, however, that an analysis for peroxidase activity could indicate the survivability of a grass after prolonged inundation. Determination of the electrophoretic pattern of peroxidase of non-inundated and inundated grasses may be a diagnostic technique in the survival assay of grasses.

Germination and seedling survival

Initially grass seeds were planted on sand, watered and set aside until seedlings reached 4-5 cm (approximately ten days after germination). However, only a few seeds of boreal red fescue, meadow foxtail and sydsport bluegrass germinated, so few seedlings were produced. To determine if this poor germination was due to the seed or the technique, a series of seed germination tests were carried out. The results of the tests (Table 4) indicated that the problem was due to the seed. Probably the length of storage or the improper storage of these seeds caused the decline in seed germination (Taylorson and Hendricks 1977).

When the experiment was set up again, a larger number of boreal red fescue and meadow foxtail seeds were added to each pot to compensate for the poor germination. Sydsport bluegrass seed was not used.

The poor germination suggests that seedling vigor may also have declined. The response of these seedlings to the inundation stress, then, may not be representative of those from fresher seed.

Seedlings from the five types of seed that were used survived the inundation treatment. The leaves remained green, but the plants tended to deteriorate over the 30-day observation period. In all cases the leaves became slightly chlorotic. Also, the leaves did not grow in length nor were new leaves produced. Perhaps if the experiment had been continued, these stressed seedlings would have died.

Table 4. Summary of the seed germination experiments. The numbers represent the averages of three tests.

Grass	Seeds germinated (%) Days after experiment initiation 2	6	16	30	Total
manchar	18	67	2	0	87
boreal red fescue	0	6	7	2	15
durar fescue	8	71	9	0	88
nugget Kentucky bluegrass	0	33	38	8	79
meadow foxtail	5	22	6	2	35
sydsport bluegrass	0	1	0	1	2

CONCLUSIONS

The grass that exhibited the best survivability after being inundated for 10 days was nugget Kentucky bluegrass. Both topsoil- and gravel-grown plants survived the stress treatments. However, sydsport bluegrass grown on gravel appeared to resist the stress treatments better, but its survivability on topsoil is unknown. Meadow foxtail and manchar brome did well on topsoil when stressed, but did poorly on gravel soil. Neither of the fescues, boreal red and durar, responded well to the stress treatments. The plants either died or had severely retarded growth.

No damage assessment could be made from root transverse sections taken soon after the stress treatment. Leaf transverse sections did show stress-related damage. Chloroplastid coloration and leaf configuration after sectioning were the most obvious differences. Adversely affected plants had either yellow (chlorotic) or brown chloroplastids and these leaves were usually broken during sectioning. Plants that were not affected had green chloroplastids and remained intact when sectioned. Because the changes seen in the sectioned leaves paralleled those of the whole leaf, sectioning and microscopic examination are not necessary for determining stress damage.

Rhizome growth and new leaf generation were found in some of the stressed plants. The original leaves of several of these plants died, giving the appearance that the whole plant had died; however, the roots and rhizomes survived and were able to produce new leaves (in manchar brome, durar fescue, and nugget Kentucky bluegrass).

Malic dehydrogenase banding patterns of roots and leaves varied but could not be correlated with any of the stress treatments. Dark-treated and inundated plants exhibited similar enzyme banding patterns. Peroxidase banding patterns from roots did not appear to be significantly related to stress damage. Leaf peroxidase banding patterns did appear to be significantly related to inundation stress. The differences in banding appeared before damage could be visually observed. This test may prove to be a diagnostic tool for assaying inundation damage to plants.

Although seedlings survived a 15-day inundation period, the results of the test were inconclusive because of the suspected poor condition of the seeds.

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APPENDIX A: SELECTED BIBLIOGRAPHY OF INUNDATION-RELATED PAPERS.

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